

Teratologic Effects of Long-Term
Exposure to Diesel Exhaust Emissions (Rabbits)

WIL Research Labs., Inc.
Cincinnati, OH

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EXPOSURE TO DIESEL EXHAUST EMISSIONS (RABBITS)**

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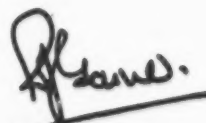
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FOREWORD

The use of diesel engines for both personal and commercial transportation is increasing, and there is very little information presently available concerning the toxicology of whole emissions from diesel exhaust.

This study was performed by WIL Research Laboratories, Inc. to contribute information as to the potential teratogenic effects of diesel exhausts when rabbits were exposed to diesel exhaust emissions. The procedures utilized in exposure and fetal evaluations were developed by the Food and Drug Administration for evaluating teratogenic effects of new drugs.



R. J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

This research project was initiated with the objective of evaluating the potential for diesel exhaust emissions to produce malformations in rabbit fetuses. The pregnant does were exposed by the inhalation route to a 10% concentration of diesel exhaust emissions in inhalation chambers on days 6 through 18 of gestation. Methods used conform to the guideline developed by the Food and Drug Administration for evaluating teratogenic effects in rabbits.

The results of the exposure of pregnant rabbits indicate that diesel exhaust emissions have no effect upon the normal development of rabbit fetuses.

Diesel exhaust emissions did not effect any of the parameters evaluated to assess maternal toxicity nor total number of fetuses born.

This report was submitted in fulfillment of Contract No. 68-03-2652 by WIL Research Laboratories, Inc. under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period of December 19, 1978 to May 25, 1979 when the work was completed.

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ABBREVIATIONS

A	=	Adipsia
B	=	Blackened Coat
D	=	Died
F	=	Female (pg. 14, 15, 16, 23, 24, 30, 31)
F	=	Fissures on back of neck (pg. 6 & 7)
G	=	Gray Coat
L	=	Left (pg. 26 & 28)
L	=	Lacrimation of eye (pg. 7)
M	=	Male
N	=	Nasal Discharge
NP	=	Not Pregnant
P	=	Pregnant
Q	=	Quarantine
R	=	Right (pg. 26 & 28)
R	=	Respiratory Congestion (pg. 7)
X	=	Anorexia

SECTION 1

INTRODUCTION

Diesel fuel has increased with the use of diesel engines in vehicular traffic. There is little information presently available concerning the toxicology of whole emissions from diesel exhaust, thus the proposal made in RFP No. C1 77-0221 was performed in pregnant laboratory animals to establish potential teratogenic effects during the period of fetal development.

The objective of this study was to test for and evaluate the potential of diesel exhaust emissions to produce malformations and/or other teratogenic effects in the unborn rabbit fetus when dams were exposed by means of inhalation during the period of organogenesis (Days 6 through 18 of gestation). The methods described herein conform to the guideline developed by FDA in 1966¹.

No malformation nor other teratogenic effects related to exposure to diesel exhaust emissions occurred in unborn rabbit fetuses when dams were exposed to a 10% concentration of diesel exhaust by means of inhalation during the period of organogenesis.

¹Guidelines for Reproductive Studies for Safety Evaluation of Drugs for Human Use, January 1966, FDA.

SECTION 2

EXPERIMENTAL DESIGN

TEST MATERIAL

The test material used in this study was diesel exhaust emissions generated by a diesel engine located at the EPA Center Hill Laboratory, Cincinnati, Ohio. As a control, clean air was administered by the same means in chambers also located at EPA Center Hill Laboratory. The first day of inhalation exposure was January 8, 1979.

TREATMENT PREPARATION AND ADMINISTRATION

A ratio of ten percent diesel exhaust emission to ninety percent clean air was administered to the test group (Group 2). Inhalation was the route of administration in chambers 4 1/2 feet square and 5 feet square. The control group (Group 1) was also exposed in 4 1/2 feet and 5 feet square inhalation chambers with 100% clean air. The Center Hill EPA laboratory was responsible for maintaining the proper exposure rates, monitoring procedures and care of the laboratory animals during the exposure period.

ANIMALS - REQUISITION AND CARE

Forty-two female New Zealand albino rabbits and eight male New Zealand albino rabbits of sexual maturity were obtained from Buckshire Corporation, Perkasio, Pennsylvania. One male New Zealand albino rabbit was obtained from Pel Freez Laboratories, Rogers, Arkansas.

Due to limited chamber space at the time, two groups, one of 22 rabbits and one of 20 rabbits were exposed approximately three months apart. The first twenty-two (22) female rabbits arrived on December 19, 1978; the second group of twenty (20) female rabbits arrived on March 7, 1979 and March 23, 1979. One (1) male rabbit arrived on December 15, 1977. Four (4) additional male rabbits arrived on January 12, 1978; the remaining four (4) male rabbits arrived on December 21, 1978.

All rabbits were examined prior to selection for this study and only those animals determined to be in good health were selected for testing. During the quarantine period the animals were permanently identified by ear tags.

All animals were housed in AALAC approved type and size cages during the quarantine period and the nonexposure period. Purina Lab Rabbit Chow and fresh tap water were available *ad libitum* during the quarantine and nonexposure periods. The animal care procedures for the female rabbits were the responsibility of WIL Research Laboratories, Inc., during the quarantine period and Days 0 through 5, 18 through 29 of gestation.

The animal care for the male rabbits was the responsibility of WIL Research Laboratories for the entire duration of the study. All animal care procedures used adhered to the principles defined in the Guide For Laboratory Animal Facilities and Care and the Animal Welfare Act and Amendments. The EPA Center Hill Laboratory, Cincinnati, Ohio, was responsible for the animal care of the female animals during the exposure period which was Days 6 through 18 of gestation. Following the exposure time in the chambers at the EPA facilities the rabbits remained in individual cages, at which time clean air was administered. The chamber rooms utilized a 12-hour light cycle during the animals' daily routines.

DOSAGE SELECTION

The EPA conducted preliminary studies to determine the 10% concentration of diesel exhaust emissions in the exposure chambers. The Environmental Protection Agency personnel were responsible for assuring chamber concentrations and exposure time.

MATING PROCEDURE

The female animals were randomly selected by stratified randomization methods and placed in either the test or control group. The female rabbits were maintained in isolation for a period sufficient to establish a stable oestrus (2 to 3 weeks in the absence of a male or other stimulation). Nine sexually mature male rabbits were untreated throughout the study and provided the sperm for breeding by artificial insemination. A collection of seminal fluid by means of an artificial vagina was obtained from as many males that would be sufficient to inseminate four rabbits per day. The sperm of the animals was pooled in a clean collection bottle and kept at a temperature of 38°C to 40°C by means of a water bath. It was then checked for motility and viability and rechecked in the same manner after each insemination. The female rabbits were then inseminated with 0.25 to 0.5 ml of the pooled sperm followed by an intravenous injection of a pituitary lutenizing hormone (PLH Armour) to insure ovulation. The day of insemination is considered to be Day 0 of gestation.

TREATMENT AND DOSING SCHEDULE

The female rabbits were exposed on Days 6 through 18 of gestation for 8 hours per day. Group 1 was exposed to 100% clean air while Group 2 was exposed to the 10% diesel exhaust emissions and 90% clean air.

OBSERVATIONS DURING GESTATION PERIOD

All female rabbits were examined daily for signs of toxicity, general health, and behavior. Each dam was weighed on Days 0, 6 through 18, and 29 of gestation. All males were observed daily and weighed weekly from the first day of insemination until the last C-section.

OBSERVATIONS AT TIME OF LAPAROTOMY

The female rabbits were terminated on Day 29 of gestation with sodium pentobarbital administered into the marginal ear vein. Cesarean section and gross necropsy of each female was witnessed by a pathologist. The following observations were made:

1. Total number of fetuses.
2. Total number of live and dead fetuses.
3. Number of resorbed or resorbing fetuses.
4. Number of implant sites.
5. Number of corpora lutea.
6. Distribution of fetuses within the uterine horns.
7. Any remarkable gross pathology in the dam.
8. Examination of the fetuses as removed from the uterus:
 - a. external conformation and anomalies.
 - b. individual body weight for each fetus and total litter weight.
 - c. sex of each fetus

The last cesarean section was performed on May 1, 1979.

FETAL EXAMINATION

Each fetus was examined by a teratologist for external anomalies of the head, mouth, dorsal and ventral aspects of the body, limbs and tail. One-third of the fetuses were placed in Bouin's fixative and examined for gross internal anomalies by careful Wilson Sectioning.² The remaining two-thirds of the fetuses were placed in 70% Ethyl Alcohol and examined for skeletal anomalies following preparation of the skeletons by the Alizarin Red S procedure³. Fetal examinations were completed on May 25, 1979.

²"Environment and Birth Defects," James G. Wilson, Children's Hospital Research Foundation, University of Cincinnati College of Medicine, 1973.

³"Pathology and Toxicology, Technique Manual," edited by L. Z. Saunders; Research and Development Division, Smith, Kline and French Laboratories, Philadelphia, Pennsylvania, 1969, pages 1-13.

SECTION 3

RESULTS

GENERAL OBSERVATIONS

The female rabbits were examined daily for signs of maternal toxicity and behavioral changes during the quarantine and nonexposure periods.

In the clean air control group one female died on the 22nd day of pregnancy after having been returned to WIL Research Laboratories. This female rabbit was grossly necropsied by a veterinary pathologist, Dr. Fred W. Sigler. It was noted that her lungs were consolidated. The diagnosis was Pasteurellosis. A copy of the documented report can be found in Appendix A. There were three rabbits which exhibited fissures on the back of their necks after the exposure period. An additional rabbit exhibited the same fissure on Day 17 of gestation. These observations were attributed to temperature or humidity irregularity. All other rabbits in this group appeared normal throughout the study.

The female rabbits of the test group did not show any change in behavior or signs of toxicity during the study. One female of this group was found dead on Day 17 of pregnancy at the EPA facility. This female rabbit was returned to WIL and a veterinary pathologist, Dr. Fred W. Sigler, performed the necropsy. The diagnosis of death was toxemia resulting from intestinal infraction produced by traumatic herniation through the left abdominal wall. The uterus was removed and 13 prenatal fetuses were recovered. These fetuses and placentas were preserved in Bouin's. A copy of the documented report can be found in Appendix B. One test rabbit exhibited fissures on back of the neck on Day 23 of gestation (after having been returned to WIL).

Throughout the exposure period (Days 6-18 of gestation), rabbits coats in the diesel fuel group were blackened by the emissions. However, it was noted that after being returned to WIL the rabbits began to clean themselves, and in most cases only their heads remained gray. From this observation we can only conclude that some particulate matter was ingested by the rabbits. How this affected the rabbits or fetuses cannot be determined. All daily observations from quarantine to the day of cesarean section for each group can be found on Tables 1 and 2.

Male rabbits used for the insemination process were also observed daily during the study. One male (395) had a 2 cm x 2 cm hairless region on the right jaw for four days. On the fifth day hair began to return to the site. (All males at this time were treated for ear mites with mineral oil.) Thirty-eight days after the hair had grown back, this rabbit had two large abscesses near the mandibular lymph nodes. This area was surgically drained and locally applied with organic iodine by a veterinarian, Dr. Vincent Chaffee. Twenty days later, abscesses reappeared. Since this male rabbit did not overcome the infection, it was sacrificed five days later. A documented copy of the reports can be found in Appendices C, D & E. Male rabbit (398) was sacrificed because of his lack of ability to supply semen for insemination.

TABLE 1
DAILY OBSERVATIONS OF DAMS WHICH WERE EXPOSED
TO CLEAN AIR (GROUP 1 CONTROL)

	Q	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
370																																
371																																
373	N		N		N	N		N				N		N		N		N	N	N		N		N				N	F	F	N	
374																																
375	N	N	NXA	NXA	NXA	N	N	N	N		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
376																																
377	N		N			N							N	N				N	N			N		N					N	N	N	
378																							F	F	F	F	F	F	F	F	F	
379	N		N		N	N		N	N		N	N	N	N							N	N	F	F	F	F	F	F	F	F	F	
380	N							N	N		N											N	F	F	F	F	F	F	F	F	F	
381																																
103																	N			N												
104	N		N		N	N		N				N				N																
105																																
106																																
107	N																															
108	N																															
109																					A											
110	N		N																												N	N
111	N																													N	N	N
124																																

Q - Quarantine

N - Nasal discharge

F - Fissures on back of neck

A - Anorexia

D - Died

Q - Quarantine
N - Nasal discharge
F - Fissures on back of neck
A - Adiposa
X - Anorexia
D - Died

TABLE 2

DAILY OBSERVATIONS OF DAMS WHICH WERE EXPOSED
TO DIESEL EXHAUST INHALATION (GROUP 2 TEST)

	DAYS OF GESTATION																														
Q	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
382								B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	G	G	G	G*
383	N					N	NB	NB	NB	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	NB	B	G	G	G	G	G*
385	NL	N	L	L	L	L	NRB	LB	LB	LB	B	B	NB	NB	NB	NB	NB	B	B	B	B	NB	NB	NB	NB	NB	NB	NG	NG	G	G
386						N	NB	NBR	NB	NB	NB	NB	NB	NB	NB	NB	NB	B	B	B	B	B	B	B	B	B	G	G	G	G*	G*
387	N	N	N	N	N	N	NB	NB	NB	NB	B	B	B	B	B	NB	NB	B	B	NAB	NB	NAB	NB	NB	NB	NG	NG	NG	NG*	NG*	NG*
388	N	N		N			NB	NB	B	B	B	B	B	B	B	B	B	B	B	B	B	NB	B	B	B	NG	G	G	G*	G*	NG*
389	N	N	N	L	N	L	N	B	B	B	L	B	B	LB	LNB	B	LNB	LNB	LNB	LNB	NLG	LG	LNG	G	G	NG	LG	LG	NG	G*	G*
390	L						B	B	B	B	B	B	B	B	B	B	B	B	B	B	NB	G	G	FG	FG	FG	FG	FG	FG	FG	FG
392	N						B	B	B	B	B	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB	NG	NG	NG	NG	NG	G	NG	NG	NG	NG
393	N	N	N	N	N	N	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB	B	B	B	NG	NG	NG	NG*	NG*	NG*	NG*	NG*	NG*	NG*	NG*	NG*
394	L						B	B	B	B	B	B	B	NB	B	B	B	B	B	B	G	G	G*	G*	G*	G*	G*	G*	G*	G*	G*
112							B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	N	ANG	ANG*	G*	G*	G*	G*	G*	G*	G*
113							B	B	NB	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	G	G*	G*	G*	G*	G*	G*
114	N						B	B	B	B	B	B	B	B	B	B	B	B	B	NB	AB	AB	B	B	B	G	G**	G**	G**	G**	G**
115	N						B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
116	N						B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B*	B*	B*	B*	B*	B*	B*	B*	B*	B*
117							B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	G	G	G*	G*	G*	G*	G*	G*	G*
118							B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	NB	G	G	G	G	G	GL	G	G
119							B	B	B	B	B	B	B	B	B	B	B	AB	B	B	G	G	G*	G*	G*	G*	G*	G*	G*	G*	G*
120	LN	L	NL	N	L	L	B	B	B	B	B	B	B	B	B	B	B	BN	B	B	G	XAG	G	G	G*	G*	G*	NG*	NG*	NG*	NG*
125							B	B	B	B	B	B	B	B	B	B	NRB	NRB	NRB	NRB	G	G	G	G	G	G	G	G	G	G	G

Q - Quarantine N - Nasal discharge X - Anorexia * - Head
 B - Blackened coat L - Laceration A - Adipsia ** - Head and along back
 G - Gray coat R - Respiratory congestion F - Fissures on back of neck D - Died

Male rabbit (433) refused to eat or drink and was shedding hair. Seven days later, male rabbit (433) was administered mineral oil by oral gavage. Dr. Vincent Chaffee performed this procedure. The next day, the animal died. Necropsy revealed a hair ball (15cm x 10cm x 7.5cm) lodged in the stomach and partially in the esophagus.

Because several other rabbits were also shedding hair, they were moved from the conventional animal room to a bio-clean animal room. This improved the coats of the animals and all appeared normal.

Male rabbit (435) died during the study. Its lungs were reddened, kidneys were pitted and mottled externally. A thick white discharge was secreted from both eyes and nose.

BODY WEIGHTS OF ADULT MALE AND FEMALE RABBITS

Female rabbits exposed to diesel exhaust emissions (Group 2) experienced no reduction in body weight gain compared to female rabbits exposed to clean air (Group 1). (Tables 3 and 4)

Male rabbits were not exposed to diesel exhaust emissions. They gained weight at a normal rate throughout the course of the project. Male rabbits that died during the course of the project experienced no weight loss. (Table 5)

FERTILITY - MATING DATA FOR ARTIFICIAL INSEMINATION

The individual mating for females in Groups 1 and 2 is presented in Table 6. The fertility index for the females for each group was calculated using the following formula:

$$\text{Fertility Index} = \frac{\text{No. Pregnant Females}}{\text{No. Females Mated}} \times 100$$

Group 1 females exposed to clean air had a conception rate of 95% (20 of 21 animals were pregnant), and the conception rate of females exposed to diesel exhaust was 90% (19 of 21 animals were pregnant).

LAPAROTOMY DATA

Diesel exhaust emissions produced no malformations or other teratogenic effects in the unborn rabbit fetus.

All rabbit pups were examined at the time of Cesarean section. The occurrence of gross abnormalities in both Group 1 and Group 2 were within normal limits. (Table 7)

The number of live and dead fetuses, number of resorptions, number of implantation sites and corpora lutea are presented in Tables 8 and 9. The early fetal deaths reported for dam number 1213-120 (Group 2) is considered to be a litter effect. None of the above parameters were altered following exposure to diesel exhaust emissions compared to controls.

The sex distribution, total number of fetuses, average fetal weight, and group fetal weight are reported in Tables 10 and 11. None of these parameters were altered following exposure to diesel exhaust emissions compared to controls.

TABLE 3 - INDIVIDUAL AND MEAN BODY WEIGHTS (IN KILOGRAMS) FOR DAMS WHICH WERE EXPOSED TO CLEAN AIR (GROUP 1 - CONTROL)

Rabbit Number	Day 0	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 29	% Body Weight Increase	P* NP**
1213-370	3.20	3.47	3.46	3.41	3.36	3.44	3.37	3.41	3.40	3.53	3.44	3.38	3.46	3.49	***	—	NP
1213-371	3.60	3.57	3.61	3.67	3.54	3.66	3.56	3.71	3.62	3.76	3.83	3.79	3.85	3.90	3.80	6	P
1213-373	3.75	3.66	3.73	3.70	3.76	3.72	3.80	3.70	3.80	3.84	3.82	3.81	3.80	3.80	4.05	8	P
1213-374	3.55	3.70	3.73	3.64	3.71	3.63	3.72	3.63	3.76	3.86	3.80	3.84	3.84	3.80	4.05	14	P
1213-375	3.30	3.60	3.30	3.25	3.11	3.15	3.15	3.17	3.15	3.25	3.26	3.30	3.33	3.33	3.47	5	P
1213-376	3.20	3.31	3.23	3.23	3.23	3.20	3.16	3.30	3.31	3.34	3.54	3.55	3.42	3.42	3.74	17	P
1213-377	3.20	3.10	3.05	3.26	3.31	3.35	3.37	3.33	3.41	3.83	3.37	3.44	3.43	3.43	3.55	11	P
1213-378	3.55	3.71	3.50	3.64	3.40	3.42	3.44	3.44	3.53	3.72	3.77	3.84	3.72	3.83	3.75	6	P
1213-379	3.45	3.45	3.55	3.50	3.53	3.48	3.50	3.41	3.52	3.62	3.66	3.63	3.69	3.70	4.02	17	P
1213-380	3.70	3.52	3.79	3.76	3.78	3.77	3.61	3.77	3.84	3.83	3.96	3.87	3.94	3.91	3.94	6	P
1213-381	3.60	3.73	3.75	3.80	3.75	3.80	3.84	3.80	3.83	3.97	3.89	3.89	3.90	3.92	4.05	13	P
1213-103	4.10	4.20	4.20	4.04	4.15	4.11	4.05	4.12	4.16	4.13	4.10	4.12	4.05	4.16	4.20	2	P
1213-104	2.80	3.00	2.95	2.93	3.01	2.97	3.03	3.05	3.08	3.14	3.15	3.19	3.17	3.16	3.45	23	P
1213-105	3.65	3.79	3.79	3.82	3.76	3.83	3.76	3.77	3.82	3.87	3.92	3.80	3.81	3.86	4.15	14	P
1213-106	3.20	3.22	3.30	3.27	3.28	3.25	3.27	3.28	3.26	3.32	3.32	3.23	3.24	3.30	3.50	9	P
1213-107	3.35	3.56	3.54	3.54	3.49	3.57	3.66	3.62	3.67	3.75	3.68	3.74	3.75	3.46	4.00	19	P
1213-108	3.40	3.53	3.54	3.48	3.46	3.47	3.44	3.46	3.52	3.58	3.45	3.51	3.50	3.56	3.80	12	P
1213-109	3.45	3.65	3.54	3.56	3.52	3.58	3.57	3.59	3.58	3.59	3.60	3.58	3.59	3.69	3.90	13	P
1213-110	3.95	4.29	3.97	4.03	4.02	4.02	4.06	4.09	4.11	4.13	4.13	4.17	4.17	4.21	4.45	13	P
1213-111	3.50	3.58	3.45	3.45	3.40	3.39	3.51	3.48	3.32	3.31	3.43	3.44	3.49	3.44	3.70	6	P
1213-124	3.75	3.81	3.77	3.77	3.82	3.77	3.76	3.80	3.78	3.83	3.80	3.79	3.83	3.86	4.05	8	P
Mean	3.49	3.60	3.56	3.56	3.54	3.55	3.56	3.57	3.59	3.68	3.66	3.66	3.67	3.68	3.70	11	

* Pregnant

** Not Pregnant

*** Rabbit found dead on 22nd day.

TABLE 4 - INDIVIDUAL AND MEAN BODY WEIGHTS (IN KILOGRAMS) FOR DAMS WHICH WERE EXPOSED TO DIESEL EXHAUST EMISSIONS (GROUP 2 - TEST)

Rabbit Number	Day 0	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 29	* Body Weight Increase	P ^{••} NP ^{•••}
1213-382	3.50	3.76	3.76	3.75	3.77	3.73	3.69	3.76	3.60	3.75	3.74	3.76	3.72	3.73	4.00	14	NP
1213-383	3.60	3.61	3.53	3.60	3.41	3.60	3.56	3.67	3.57	3.72	3.74	3.72	3.72	3.74	3.95	10	P
1213-385	3.70	3.80	3.76	3.75	3.80	3.68	3.77	3.77	3.80	3.82	3.80	3.77	3.80	3.75	3.95	7	P
1213-386	3.40	3.43	3.31	3.29	3.37	3.46	3.40	3.43	3.10	3.51	3.56	3.55	3.50	3.55	3.77	11	P
1213-387	3.65	3.84	3.85	3.81	3.79	3.81	3.80	3.85	3.62	3.70	3.80	3.83	3.80	3.74	4.00	10	NP
1213-388	3.80	3.93	3.90	4.15	3.93	3.89	3.85	3.96	3.96	3.96	4.50	4.51	4.56	4.16	4.29	13	P
1213-389	3.05	3.22	3.24	3.28	3.22	3.29	3.19	3.20	3.26	3.34	3.33	3.37	3.37	3.31	3.55	16	P
1213-390	3.80	3.86	3.78	3.92	3.84	3.83	3.92	3.91	3.83	3.79	3.77	3.71	3.88	4.10	3.85	13	P
1213-392	3.85	3.40	3.40	3.37	3.40	3.47	3.44	3.50	3.54	3.55	3.63	3.58	3.59	3.57	3.75	-3***	P
1213-393	3.65	3.60	3.72	3.72	3.70	3.66	3.72	3.73	3.78	3.80	3.75	3.77	3.73	3.76	3.95	8	P
1213-394	3.50	3.72	3.70	3.70	3.66	3.69	3.70	3.73	3.86	3.87	3.81	3.87	3.85	3.85	3.98	14	P
1213-112	3.15	3.25	3.44	3.40	3.37	3.48	3.37	3.48	3.45	3.47	3.56	3.55	3.59	3.47	3.65	16	P
1213-113	3.50	3.67	3.62	3.59	3.53	3.54	3.57	3.61	3.60	3.61	3.63	3.62	3.67	3.65	3.75	7	P
1213-114	3.55	3.68	3.74	3.74	3.73	3.64	3.71	3.67	3.67	3.64	3.64	3.74	3.73	3.75	3.95	11	P
1213-115	3.60	3.66	3.82	3.75	3.86	3.71	3.82	3.84	3.73	3.80	3.69	3.57	3.47****	—	—	—	P
1213-116	3.70	3.85	3.85	3.94	3.89	3.97	4.01	4.00	4.01	4.11	3.91	4.07	4.00	3.99	4.40	19	P
1213-117	3.30	3.41	3.34	3.32	3.29	3.39	3.33	3.43	3.42	3.45	3.43	3.44	3.50	3.39	3.65	11	P
1213-118	3.85	3.87	3.87	3.84	3.81	3.82	3.81	3.81	3.80	3.83	3.78	3.80	3.93	3.90	3.85	0	P
1213-119	4.15	4.25	4.29	4.25	4.36	4.36	4.34	4.37	4.40	4.31	4.40	4.40	4.30	4.43	4.70	13	P
1213-120	3.30	3.47	3.57	3.57	3.57	3.55	3.57	3.59	3.57	3.62	3.68	3.71	3.71	3.71	4.00	21	P
1213-125	4.00	4.21	4.23	4.22	4.20	4.14	4.20	4.23	4.10	4.21	4.24	4.20	4.29	4.22	4.35	9	P
Mean	3.60	3.69	3.70	3.71	3.69	3.70	3.70	3.74	3.70	3.76	3.78	3.79	3.80	3.79	3.97	11	

* Pregnant

** Not Pregnant

*** 3% body weight decrease due to water problem between Day 0 and Day 6 of gestation.

****Rabbit found dead, see results.

TABLE 5 - WEEKLY BODY WEIGHTS (IN KILOGRAMS) FOR MALE RABBITS
USED IN THE ARTIFICIAL INSEMINATION OF THE FEMALE RABBITS

Animal No.	WEEK												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1213-435	4.90	5.00	5.05	4.95	5.05	5.05	5.05	5.25 ¹					
1213-433	4.60	4.70	4.65	4.80	4.85	4.85	4.95 ¹						
1213-339	4.80	4.80	4.80	4.90	5.05	4.90	4.90	4.95	4.90	4.70	4.75	4.85	4.70
1213-434	4.50	4.55	4.60	4.50	4.60	4.60	4.45	4.55	4.55	4.65	4.70	4.70	4.65
1213-440	5.30	5.25	5.35	5.25	5.40	5.45	5.45	5.35	5.30	5.35	5.40	5.35	5.35
1213-395	3.55	3.80	3.80	3.80	3.95	3.95	3.95 ²						
1213-396	4.00	4.00	4.05	4.10	4.20	4.20	4.25	4.25	3.90	4.10	4.20	4.20	4.25
1213-397	3.95	3.95	4.00	4.15	4.35	4.50	4.60	4.90	4.90	5.00	5.00	5.10	5.10
1213-398	4.60	4.80	4.90	5.05	5.00	4.95	5.05 ³						
Mean	4.47	4.54	4.58	4.61	4.72	4.72	4.74	4.88	4.71	4.76	4.81	4.84	4.81

¹ Found dead (see results).

² Sacrificed due to illness (see results).

³ Sacrificed because buck did not respond to procedure involving collection of semen (see results).

TABLE 6 - MATING SCHEDULE

Group 1		Group 2	
Doe Number	Pooled Semen Bucks No.	Doe Number	Pooled Semen Bucks No.
1213-370	1213-434, 433	1213-382	1213-435, 395
1213-371	1213-434, 433	1213-383	1213-435, 395
1213-373	1213-339, 395	1213-385	1213-339, 395
1213-374	1213-339, 395	1213-386	1213-339, 395
1213-375	1213-434, 435	1213-387	1213-396, 433
1213-376	1213-434, 435	1213-388	1213-396, 433
1213-377	1213-339, 395, 434	1212-389	1213-339, 395, 434
1213-378	1213-339, 395, 434	1213-390	1213-339, 395, 434
1213-379	1213-435, 440, 339, 434	1213-392	1213-435, 440, 339, 434
1213-380	1213-435, 440, 339, 434	1213-393	1213-435, 440, 339, 434
1213-381	1213-339, 434, 435	1213-394	1213-339, 434, 435
1213-103	1213-440, 397, 434, 435	1213-112	1213-440, 397, 434, 435
1213-104	1213-440, 397, 434, 435	1213-113	1213-440, 397, 434, 435
1213-105	1213-339, 440, 397, 434, 435	1213-114	1213-339, 440, 397, 434, 435
1213-106	1213-339, 440, 397, 434, 435	1213-115	1213-339, 440, 397, 434, 435
1213-107	1213-339, 440, 397, 435	1213-116	1213-339, 440, 397, 435
1213-108	1213-339, 440, 397, 435	1213-117	1213-339, 440, 397, 435
1213-109	1213-339, 440, 397, 396, 434, 435	1213-118	1213-339, 440, 397, 396, 434, 435
1213-110	1213-339, 440, 397, 396, 434, 435	1213-119	1213-339, 440, 397, 396, 434, 435
1213-111	1213-339, 440, 434, 397	1213-120	1213-339, 440, 434, 397
1213-124	1213-339, 440, 434, 397	1213-125	1213-339, 440, 434, 397

TABLE 7

GROSS FETAL EXAMINATION AT TIME OF LAPAROTOMY

<u>Dam Number</u>	<u>Fetus No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Gross Observations</u>
1213-370*		Clean Air		
1213-371		Clean Air		All fetuses appeared normal.
1213-373	5	Clean Air	F	Fetus born dead.
1213-374		Clean Air		All fetuses appeared normal.
1213-375		Clean Air		All fetuses appeared normal.
1213-376	6	Clean Air	M	Fetus born dead. Fetus was light brown in color and had short limbs and was smaller in size compared to other fetuses in the litter.
	8	Clean Air	F	Abdomen and occiput enlarged.
	11	Clean Air	F	Abdomen and occiput enlarged, short limbs and cleft palate.
1213-377	2	Clean Air	F	Cleft palate.
	4	Clean Air	F	Fetus born dead. Body was gelatinous and fetus was slightly smaller than other fetuses in the litter.
1213-378		Clean Air		All fetuses appeared normal.
1213-379		Clean Air		All fetuses appeared normal.
1213-380		Clean Air		All fetuses appeared normal.
1213-381		Clean Air		All fetuses appeared normal.
1213-103		Clean Air		All fetuses appeared normal.
1213-104		Clean Air		All fetuses appeared normal.

*Not pregnant.

Continued...

TABLE 7

GROSS FETAL EXAMINATION AT TIME OF LAPARTOMY (Continued)

<u>Dam Number</u>	<u>Fetus No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Gross Observations</u>
1213-105		Clean Air		All fetuses appeared normal.
1213-106		Clean Air		All fetuses appeared normal.
1213-107	6	Clean Air	F	Fetus born dead. Fetus was very small in size compared to others in the litter. Body was brown in color with subcutaneous fluid. Head appeared to be abnormal, and there was no eye development. Placenta was granular.
1213-108		Clean Air		All fetuses appeared normal.
1213-109		Clean Air		All fetuses appeared normal.
1213-110	12	Clean Air	F	Fetus born dead. Body was transparent with subcutaneous fluid. Placenta was granular.
1213-111	11	Clean Air	*	Very early fetal death.
1213-124	10	Clean Air	F	Left eye was not fully developed.
1213-382**		Diesel Exhaust		
1213-383		Diesel Exhaust		All fetuses appeared normal.
1213-385		Diesel Exhaust		All fetuses appeared normal.
1213-386		Diesel Exhaust		All fetuses appeared normal.
1213-387**		Diesel Exhaust		

*Sex could not be determined.

**Not pregnant.

Continued...

TABLE 7

GROSS FETAL EXAMINATION AT TIME OF LAPAROTOMY (Continued)

<u>Number</u>	<u>No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Gross Observation</u>
1213-388	2	Diesel Exhaust	M	Fetus born dead. Fetus smaller in size compared to other fetuses in the litter. Fetus was light brown in color and appeared to have no brain.
1213-389		Diesel Exhaust		All fetuses appeared normal.
1213-390		Diesel Exhaust		All fetuses appeared normal.
1213-392		Diesel Exhaust		All fetuses appeared normal.
1213-393		Diesel Exhaust		All fetuses appeared normal.
1213-394		Diesel Exhaust		All fetuses appeared normal.
1213-112		Diesel Exhaust		All fetuses appeared normal.
1213-113		Diesel Exhaust		All fetuses appeared normal.
1213-114	5	Diesel Exhaust	M	Fetus born dead.
1213-115*				
1213-116	5	Diesel Exhaust	F	Fetus born dead. Body was dark grey in color with subcutaneous fluid. Eyes appeared to be underdeveloped. Placenta was granular.
1213-117	6	Diesel Exhaust	F	Fetus born dead. Body was transparent with subcutaneous fluid. There appeared to be no eye development.
	8	Diesel Exhaust	F	Fetus born dead. Body was transparent with subcutaneous fluid. There appeared to be no eye development.

* Dam was found dead on Day 17 of exposure, 13 fetuses were in prenatal development (see results).

Continued...

TABLE 7

GROSS FETAL EXAMINATION AT TIME OF LAPAROTOMY (Continued)

<u>Dam Number</u>	<u>Fetus No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Gross Observations</u>
1213-118		Diesel Exhaust		All fetuses appeared normal.
1213-119		Diesel Exhaust		All fetuses appeared normal.
1213-120	4	Diesel Exhaust	*	Very early fetal death.
	5	Diesel Exhaust	F	Very early fetal death.
	6	Diesel Exhaust	M	Very early fetal death.
	8	Diesel Exhaust	F	Very early fetal death.
	9	Diesel Exhaust	F	Very early fetal death.
	10	Diesel Exhaust	M	Very early fetal death.
1213-125		Diesel Exhaust		All fetuses appeared normal.

*Sex could not be determined.

TABLE 8 - FETAL DATA FOR DAMS WHICH WERE EXPOSED TO CLEAN AIR (GROUP 1 - CONTROL)
NUMBER OF FETUSES VIABLE AND DEAD, EARLY RESORPTIONS, IMPLANTATION SITES AND CORPORA LUTEA

Dam Number	Viable Fetuses			Dead Fetuses			Resorptions			Implantation Sites			Corpora Lutea		
	Left Horn	Right Horn	Total	Left Horn	Right Horn	Total	Left Horn	Right Horn	Total	Left Horn	Right Horn	Total	Left Horn	Right Horn	Total
1213-370*	5	5	10	0	0	0	0	0	0	5	5	10	5	5	10
1213-371	0	3	3	0	1	1	2	0	2	2	4	6	4	11	15
1213-373	5	4	9	0	0	0	1	0	1	6	4	10	6	4	10
1213-374	4	5	9	0	0	0	0	0	0	4	5	9	7	6	13
1213-375	6	4	10	1	0	1	0	0	0	7	4	11	6	**	**
1213-376	7	3	10	1	0	1	0	1	1	8	4	12	8	4	12
1213-377	4	6	10	0	0	0	0	0	0	4	6	10	5	7	12
1213-378	6	4	10	0	0	0	0	0	0	6	4	10	6	5	11
1213-379	4	6	10	0	0	0	0	1	1	4	7	11	4	8	12
1213-380	4	8	12	0	0	0	0	0	0	4	8	12	5	9	14
1213-381	1	2	3	0	0	0	1	0	1	2	2	4	10	3	13
1213-103	4	7	11	0	0	0	0	0	0	4	7	11	6	10	16
1213-104	4	5	9	0	0	0	0	0	0	4	5	9	5	5	10
1213-105	2	7	9	0	0	0	1	0	1	3	7	10	3	7	10
1213-106	7	3	10	1	0	1	0	0	0	8	3	11	8	3	11
1213-107	4	4	8	0	0	0	0	0	0	4	4	8	5	4	9
1213-108	3	6	9	0	0	0	0	0	0	3	6	9	3	6	9
1213-109	6	6	12	0	1	1	0	0	0	6	7	13	6	7	13
1213-110	5	8	13	0	1	1	0	0	0	5	9	14	5	9	14
1213-111	7	5	12	0	0	0	0	0	0	7	5	12	7	5	12
1213-124															

* Rabbit was not pregnant.
** Right ovary was lost during necropsy procedure.

TABLE 9 - FETAL DATA FOR DAMS WHICH WERE EXPOSED TO DIESEL EXHAUST EMISSIONS (GROUP 2 - TEST)
NUMBER OF FETUSES VIABLE AND DEAD, EARLY RESORPTIONS, IMPLANTATION SITES AND CORPORA LUTEA

Dam Number	Viable Fetuses			Dead Fetuses			Resorptions			Implantation Sites			Corpora Lutea		
	Left Horn	Right Horn	Total	Left Horn	Right Horn	Total	Left Horn	Right Horn	Total	Left Horn	Right Horn	Total	Left Horn	Right Horn	Total
1213-382*															
1213-383	7	2	9	0	0	0	0	0	0	7	2	9	7	3	10
1213-385	5	3	8	0	0	0	0	0	0	5	3	8	5	4	9
1213-386	4	6	10	0	0	0	1	0	1	5	6	11	5	6	11
1213-387*															
1213-388	6	4	10	1	0	1	0	0	0	7	4	11	7	5	12
1213-389	2	5	7	0	0	0	0	0	0	2	5	7	3	6	9
1213-390	3	7	10	0	0	0	0	1	1	3	8	11	4	7	11
1213-392	6	6	12	0	0	0	1	1	2	7	7	14	8	8	16
1213-393	4	1	5	0	0	0	0	3	3	4	4	8	4	5	9
1213-394	5	6	11	0	0	0	0	0	0	5	6	11	6	6	12
1213-112	9	1	10	0	0	0	0	0	0	9	1	10	12	4	16
1213-113	3	3	6	0	0	0	0	0	0	3	3	6	4	5	9
1213-114	3	8	11	0	1	1	0	0	0	3	9	12	3	9	12
1213-115**															
1213-116	5	7	12	1	0	1	0	0	0	6	7	13	6	7	13
1213-117	4	3	7	0	2	2	0	0	0	4	5	9	4	5	9
1213-118	2	3	5	0	0	0	2	0	2	4	3	7	4	5	9
1213-119	4	8	12	0	0	0	0	0	0	4	8	12	5	8	13
1213-120	4	3	7	3	3	6	0	0	0	7	6	13	7	7	14
1213-125	6	1	7	0	0	0	0	0	0	6	1	7	7	5	12

* Rabbit was not pregnant.

** Rabbit found dead on 17th day of pregnancy, see results.

TABLE 10 - FETAL DATA FOR DAMS WHICH WERE EXPOSED TO CLEAN AIR (GROUP 1 - CONTROL)
SEX DISTRIBUTION, AVERAGE FETAL WEIGHT (g), GROUP FETAL WEIGHT (g) AND FETUS POSITION IN UTERINE HORN¹

Dam Number	Sex Distribution		Total No. of Fetuses	Avg. Fetal Wt. (g)	Group Fetal Wt. (g)														
	Males	Females				1	2	3	4	5	6	7	8	9	10	11	12	13	14
1213-370 ²																			
1213-371	7	3	10	31.0	309.7	39.2	30.5	26.5	29.7	32.4/	23.8	29.1	25.9	34.7	37.9				
1213-373	2	1	4 ³	43.3	173.0	*	*/	46.3	39.5	35.6	51.6								
1213-374	4	5	9	41.8	376.0	46.0	46.4	**	34.4	35.4	36.6/	43.5	42.9	44.1	46.7				
1213-375	6	3	9	36.4	327.9	40.1	38.1	38.9	35.4/	31.1	29.5	37.3	39.2	38.3					
1213-376	5	6	11	33.5	335.2	39.8	36.2	33.7	32.6	28.4	11.2	27.1/	27.2	34.8	36.6	27.6			
1213-377	6	5	11	29.9	298.5	28.6	30.4	29.9	19.0	26.7	30.7	27.1	27.4/	32.5	33.6	31.6			
1213-378	5	5	10	34.8	348.3	36.6	35.9	37.2	34.6/	34.4	32.3	34.3	32.5	34.3	36.2				
1213-379	6	3	10 ³	44.5	445.1	45.0	43.6	48.6	42.6	36.1	34.8/	42.3	53.7	50.2	48.2				
1213-380	6	4	10	32.4	356.0	38.0	38.3	32.1	36.3/	34.2	29.1	34.2	37.3	*	40.3	36.2			
1213-381	6	6	12	31.7	380.5	35.1	33.2	34.1	33.1/	29.3	21.6	28.4	36.7	33.4	30.0	26.6	39.0		
1213-103	2	1	3	39.9	119.7	40.7	*/	37.0	42.0										
1213-104	6	5	11	37.2	408.8	39.5	41.5	32.9	35.6/	36.0	29.9	32.3	37.6	41.6	39.6	42.3			
1213-105	5	4	9	38.6	347.3	42.2	41.2	29.9	35.5/	36.2	37.9	43.9	34.5	46.0					
1213-106	4	5	9	36.8	331.3	*	41.0	38.1/	37.4	25.7	39.1	39.3	32.2	39.1	39.4				
1213-107	5	6	11	31.2	343.3	41.1	39.6	38.4	33.5	31.6	5.3	21.5	24.6/	38.0	44.4	25.3			
1213-108	7	1	8	45.1	360.4	49.8	47.1	36.8	39.8/	43.2	45.2	46.8	51.7						
1213-109	4	5	9	38.6	347.5	40.8	39.0	39.2/	41.7	35.8	36.8	36.1	40.3	37.8					
1213-110	7	6	13	33.0	429.3	39.3	35.3	27.9	31.5	31.2	36.6/	36.9	33.3	29.6	28.4	34.9	29.6	34.8	
1213-111	8	5	13	28.0	391.4	38.5	34.1	32.9	29.6	35.9/	27.5	18.9	21.5	19.1	31.9	.6	31.1	35.0	34.8
1213-124	6	6	12	35.3	424.1	51.1	44.2	32.4	21.6	28.3	23.1	32.2/	34.2	38.5	37.2	36.8	44.5		

¹ Positions counted from the left uterine horn to the right uterine horn.

² Rabbit died and was not pregnant.

³ Total includes fetus that could not be sexed.

* Early resorption site.

** Late resorption site.

/ Denotes cervix.

TABLE 11 - FETAL DATA FOR DAMS WHICH WERE EXPOSED TO DIESEL EXHAUST EMISSIONS (Group 2 - TEST)
SEX DISTRIBUTION, AVERAGE FETAL WEIGHT (g), GROUP FETAL WEIGHT (g) AND FETUS POSITION IN UTERINE HORN¹

Dam Number	Sex Distribution		Total No. of Fetuses	Avg. Fetal Wt. (g)	Group Fetal Wt. (g)														
	Males	Females				1	2	3	4	5	6	7	8	9	10	11	12	13	14
1213-382 ²																			
1213-383	4	5	9	40.6	365.5	41.2	45.8	40.5	40.1	38.7	36.9	40.6/	39.4	42.3					
1213-385	3	5	8	30.4	243.5	33.3	29.4	29.9	25.6	27.8/	31.5	32.2	33.8						
1213-386	6	4	10	29.8	298.0	35.6	31.0	27.6	29.0	*	26.6	24.2	28.4	28.6	33.2	33.8			
1213-387 ²																			
1213-388	7	4	11	37.0	369.9	38.5	13.4	28.9	34.2	32.4	32.2	35.4/	36.4	36.6	43.1	38.8			
1213-389	6	1	7	41.9	293.4	47.9	42.5/	43.1	33.3	35.0	41.5	50.1							
1213-390	7	3	10	32.1	352.9	40.4	42.3	36.6/	31.8	23.1	28.3	*	37.3	37.1	34.6	41.4			
1213-392	7	5	12	25.5	306.2	32.1	*	18.2	28.1	26.4	28.2	24.7/	26.5	24.4	24.3	24.4	24.3	24.6	*
1213-393	2	3	5	46.7	233.5	46.5	47.1	46.5	43.6/	*	49.8	*							
1213-394	5	6	11	36.0	395.9	44.2	36.4	31.6	32.8	24.1/	36.7	34.6	41.2	42.1	34.4	37.8			
1213-112	6	4	10	34.5	344.5	38.6	31.3	33.1	40.2	36.4	30.9	23.3	27.7	38.0/	45.0				
1213-113	3	3	6	48.5	291.1	52.5	50.5	46.9/	46.4	46.3	48.5								
1213-114	8	4	12	31.5	377.4	37.0	36.5	33.9/	19.5	18.0	22.9	33.3	32.0	34.1	34.1	36.4	39.7		
1213-115 ³																			
1213-116	6	7	13	36.4	473.3	50.1	37.5	38.1	32.5	17.5	33.6/	36.0	28.0	32.7	35.9	42.5	38.8	50.1	
1213-117	2	7	9	33.8	304.2	44.3	39.6	43.3	39.6/	44.8	9.9	35.3	6.4	41.0					
1213-118	1	4	5	46.9	234.3	*	48.8	45.1	*	47.7	50.8	41.9							
1213-119	7	5	12	39.6	475.2	54.0	49.5	38.1	37.7/	27.0	30.1	35.2	47.5	34.0	37.8	42.3	42.0		
1213-120	4	8	13 ⁴	22.4	291.1	43.2	40.7	40.3	1.9	2.0	2.3	29.2/	3.6	2.8	1.0	38.6	38.7	46.8	
1213-125	4	3	7	47.8	334.5	51.2	48.3	43.1	45.3	48.1	47.0/	51.5							

¹ Positions counted from the left uterine horn to the right uterine horn.

² Not pregnant.

³ Rabbit found dead on 17th day of pregnancy; see results.

⁴ Total includes fetus that could not be sexed.

* Early resorption site.

/ Denotes cervix.

Soft tissue examination of fetuses is presented in Table 12. No malformations directly related to diesel exhaust emission exposure were observed. All anomalies observed were considered to be slight modifications from normal or related to fetal developmental age (in early fetal deaths). The occurrence of these anomalies were within the normal limits of variation.

Skeletal examinations of fetuses are presented in Table 15. No malformations directly related to diesel exhaust emission exposure were observed. Table 15 contains a list of skeletal anomalies found in both Group 1 and Group 2. The incomplete ossification of the 5th sternbra found in 9 Group 1 litters and 6 Group 2 litters is consistent with the developmental age of the fetuses (Day 29).

No signs of maternal toxicity were observed during the gross necropsy of the dams. The lungs of diesel exhaust exposed animals were pinkish-brown in color compared to the pink color in controls. This change is attributed to a response to exposure to diesel exhaust emissions. (Table 16)

FETAL VISCERAL DATA

Table 12 presents the soft tissue examinations of fetuses. The only fetuses listed in this table are anomalous. No conclusive test material effects were noted when compared to controls.

FETAL SKELETAL DATA

Tables 13 and 14 present the mean values of the ossified elements for term rabbit fetuses at the time of skeletal examinations.

Table 15 presents the skeletal examination of the fetuses which show anomalies.

No conclusive test material effects were noted when compared to controls.

GROSS NECROPSIES

Table 16 presents the gross necropsy findings from the dams in both Group 1 and Group 2. The only significant observation made for Group 2, the diesel fuel group, was the pinkish-brown appearance of the lungs compared to the lung's pink appearance in the Group 1, clean air group. Observations at the time of necropsy were made by a veterinary pathologist, Dr. Fred Sigler.

TABLE 12
SOFT TISSUE EXAMINATION OF THE FETUSES

<u>Dam Number</u>	<u>Fetus No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Abnormality</u>
1213-373	4	Clean Air	*	Eyes were underdeveloped. Brain was of a soft consistency and difficult to distinguish. Reproductive system was undistinguishable.
1213-376	6	Clean Air	M	Eyes were underdeveloped. Brain was of a soft consistency and difficult to distinguish.
	8	Clean Air	F	Cerebral hemispheres absent.
	11	Clean Air	F	Brain dark in areas and cerebral hemispheres absent. Red substance in forebrain. Midbrain reduced in size. Cleft palate.
1213-377	4	Clean Air	F	Eyes were underdeveloped. Brain was unrecognizable due to its soft consistency.
1213-378	1	Clean Air	F	Right ovary could not be identified.
1213-105	7	Clean Air	F	There was a red substance around eyes and ventricles of the brain.
1213-106	4	Clean Air	M	There was a red substance around eyes and ventricles of brain.
	7	Clean Air	M	There was a red substance around eyes and ventricles of the brain.
1213-107	6	Clean Air	F	Eyes, olfactory bulbs, brain and intestines were undistinguishable.
1213-108	4	Clean Air	M	There was a red substance around olfactory bulbs.

* Sex could not be determined.

Continued...

TABLE 12 (Continued)
SOFT TISSUE EXAMINATIONS OF FETUSES

<u>Dam Number</u>	<u>Fetus No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Abnormality</u>
1213-111	6	Clean Air	F	Brain was hydrocephalic.
	11	Clean Air	*	
1213-388	2	Diesel Exhaust	M	Palate and eyes were incomplete. Olfactory bulbs could not be identified. Organs and vessels were malaligned. The head was microcephalic, and the brain contained a red substance. There was common ductus and an intraventricular septal defect. Liver was soft and had no definitive lobes.
1213-392	4	Diesel Exhaust	M	Right testicle was lower than left testicle.
	8	Diesel Exhaust	M	Right testicle was lower than left testicle.
1213-112	8	Diesel Exhaust	M	Brain contained a red substance.
1213-116	1	Diesel Exhaust	M	There was a red substance which appeared to be blood around the olfactory bulbs and eyes.
	3	Diesel Exhaust	F	There was a red substance around the eyes and olfactory bulbs.
	5	Diesel Exhaust	F	Eyes were underdeveloped. Olfactory bulbs, brain and liver were soft in consistency.
	6	Diesel Exhaust	M	There was a red substance around eyes and olfactory bulbs.

*Fetus too small and fragile to section; no evaluation of viscera could be made.

Continued...

TABLE 12 (Continued)
SOFT TISSUE EXAMINATIONS OF FETUSES

<u>Dam Number</u>	<u>Fetus No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Abnormality</u>
1213-117	6	Diesel Exhaust	F	Eyes were underdeveloped. Olfactory bulbs and ventricles of brain were of a soft consistency. Aorta and heart were soft thus normality of heart could not be determined. Liver was also soft but lobes appeared normal.
	8	Diesel Exhaust	F	Eyes were underdeveloped. Olfactory bulbs and brain were of a soft consistency. Liver was also soft but lobes were distinguishable.
1213-120	4	Diesel Exhaust	*	Trachea, esophagus and liver were the only recognizable internal organs.
	5	Diesel Exhaust	F	Eyes were underdeveloped. Brain was of a soft consistency. Thymus and ureters were not distinguishable.
	8	Diesel Exhaust	F	Eyes were underdeveloped. Ventricular septum and ureters could not be identified.
	9	Diesel Exhaust	F	Eyes were underdeveloped. Olfactory bulbs and brain were unrecognizable. Ductus arteriosus could not be identified. Ureters were undistinguishable.
	10	Diesel Exhaust	M	Eyes were underdeveloped. Olfactory bulbs and brain were of a soft, unrecognizable consistency. Ventricular septum could not be identified.

*Sex could not be determined.

TABLE 13
SKELETAL EXAMINATIONS OF TERM FETUSES FOR CLEAN AIR (GROUP 1 - CONTROL)
MEAN NUMBER OF OSSIFIED ELEMENTS

	371	373	374	375	376	377	378	379	380	381
Cervical	7	7	7	7	7	7	7	7	6.9	7
Thoracic	12.9	12	12.5	12.8	12.2	12.3	12.5	12.1	12.6	12.5
Lumbar	7	7	7	7	7	7	7	7	7	7
Sacral	4 ¹	4	4	4	4	4	4	4	4	4
Tail	15.4 ¹	16	15	14.3	15.5	15.4	14.5	15.1	15.3	14.8
Sternum	6	6	5.2	6	6	5.7	5.3	6	5.9	5.8
Ribs	12.8	12	12.4	12.8	12.2	12.3	12.5	12.1	12.5	12.4
Fore Digits	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R
Metacarpals	4.9 4.9	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5
Proximal	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2
1.	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3
Middle 2.	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3
3.	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3
Distal	2.6 2.7	2.5 2.5	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3
Hind Digits	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R
Metacarpals	4 4	4 4	4 4	4 4	4 4	4 4	4 4	4 4	4 4	4 4
Proximal	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3
1.	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3
Middle 2.	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3
Distal	2.9 3	3 3	3 3	3 3	3 3	3 3	3 3	3 3	2.7 2.7	3 3

¹ Two rabbits had tails partially missing due to skinning procedure; means do not reflect this.

(Continued...)

TABLE 13 (Continued)
SKELETAL EXAMINATIONS OF TERM FETUSES FOR CLEAN AIR (GROUP 1 - CONTROL)

MEAN NUMBER OF OSSIFIED ELEMENTS

	Dam Number										
	103	104	105	106	107	108	109	110	111	124	
Cervical	7	7	7	7	7	7	7	7	7	7	
Thoracic	12	12.5	12	12.5	12.5	12	12.3	12.8	12	12.3	
Lumbar	7	7	7	7	6.7	7	7	7	7	6.9	
Sacral	4	4	4	4	4	4	4	4	4	4	
Tail	16	15.5	15.3	16.3	15.5	15.3	15.5	16.2	15.7	15.9	
Sternum	6	6	6	6	5.7	5.8	5.8	5.9	5.8	5.9	
Ribs	12	12.5	12	12.6	12.5	12	12.3	12.8	12	12.4	
Fore Digits	L	L	L	L	L	L	L	L	L	L	
Metacarpals	R	R	R	R	R	R	R	R	R	R	
Proximal	5	5	4.5	5	4.7	5	5	4.9	4.7	4.9	
Middle 1.	2	2	2	2	2	2	2	2	2	2	
Middle 2.	3	3	3	3	3	3	3	3	3	3	
Middle 3.	3	3	3	3	3	3	3	3	3	3	
Distal	3	3	3	3	3	3	3	3	3	3	
Hind Digits	3	2.9	2.8	3	2.7	3	2.7	3	2.3	2.6	
Metacarpals	L	L	L	L	L	L	L	L	L	L	
Proximal	R	R	R	R	R	R	R	R	R	R	
Middle 1.	4	4	4	4	4	4	4	4	4	4	
Middle 2.	3	3	3	3	3	3	3	3	3	3	
Distal	3	3	3	3	3	3	3	3	3	3	
	3	3	2.8	3	2.8	3	2.8	3	2.7	2.8	

²One rabbit had second middle digit removed during skinning procedure; mean does not reflect this.

TABLE 14

SKELETAL EXAMINATIONS OF TERM FETUSES FOR DIESEL EXHAUST EMISSIONS (GROUP 2 - TEST)

MEAN NUMBER OF OSSIFIED ELEMENTS

	Dam Number									
	383	385	386	388	389	390	392	393	394	112
Cervical	7	7	7	7	7	6.8	7	7	7	7
Thoracic	12.7	13	13	12.7	12.3	12.5	12.9	12	12.7	12.4
Lumbar	7	7	7	7	7	7	7	7	7	7
Sacral	4	4	4	4	4 ¹	4	4	4	4 ¹	4
Tail	16	15.8	15	15.4	15 ¹	15.7	15.1 ¹	15.3	15.3 ¹	15.6
Sternum	6	6	6	6	5.8	6	6	6	5.7	6
Ribs	12.7	13	13	12.7	12.3	12.4	12.8	12.3	12.7	12.5
Fore Digits	L	L	L	L	L	L	L	L	L	L
Metacarpals	R	R	R	R	R	R	R	R	R	R
Proximal	5	5	5	5	4.8	5	4.5	4.7	5	5
1.	2	2	2	2	2	1.7	2	2	2	2
2.	3	3	3	3	3	3	3	3	3	3
3.	3	3	3	3	3	3	3	3	3	3
Middle	3	3	3	3	3	3	3	3	3	3
Distal	3	3	3	3	3	3	2.8	3	3	3
Hind Digits	L	L	L	L	L	L	L	L	L	L
Metacarpals	R	R	R	R	R	R	R	R	R	R
Proximal	4	4	4	4	4	4	4	4	4	4
1.	3	3	3	3	3	3	3	3	3	3
Middle	3	3	3	3	3	3	3	3	3	3
2.	3	3	3	3	3	3	3	3	3	3
Distal	3	3	3	3	3	3	2.6	3	3	3

¹ Rabbit's tail was partially removed due to skinning procedure; means do not reflect this.

(Continued...)

TABLE 14 (Continued)
SKELETAL EXAMINATIONS OF TERM FETUSES FOR DIESEL EXHAUST EMISSIONS (GROUP 2 - TEST)
MEAN NUMBER OF OSSIFIED ELEMENTS

	Dam Number							
	113	114	116	117	118	119	120	125
Cervical	7	7	7	7	7	7	7	7
Thoracic	12.8	12.5	12.8	12.3	12.3	12.4	12.3	12.2
Lumbar	6.3	6.9	7	7	7	6.6	7	6.8
Sacral	4	4	4	4	4	4	4	4
Tail	16	16	15.1	16.2 ¹	16 ¹	16	15.9	16.2
Sternum	6	6	5.8	5.8	6	6	6	6
Ribs	12.8	12.5	12.8	12.3	12.3	12.4	12.3	12.2
Fore Digits	L	R	L	R	L	R	L	R
Metacarpals	5	4.8	5	5	5	4.9	5	5
Proximal	2	2	2	2	2	2	2	2
1.	3	3	3	3	3	3	3	3
Middle 2.	3	3	3	3	3	3	3	3
3.	3	3	3	3	3	3	3	3
Distal	3	2.9	3	3	3	3	3	3
Hind Digits	L	R	L	R	L	R	L	R
Metacarpals	4	4	4	4	4	4	4	4
Proximal	3	3	3	3	3	3	3	3
1.	3	3	3	3	3	3	3	3
Middle 2.	3	3	3	3	3	3	3	3
Distal	3	2.9	3	3	3	3	3	3

¹ Rabbit's tail was partially removed due to skinning procedure; means do not reflect this.

TABLE 15
SKELETAL EXAMINATION OF FETUSES

<u>Dam Number</u>	<u>Fetus No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Abnormality</u>
1213-104	1	Clean Air	F	5th sternebra incomplete.
1213-105	2	Clean Air	F	5th sternebra incomplete.
	3	Clean Air	M	2nd sternebra incomplete.
	6	Clean Air	F	5th sternebra incomplete.
	9	Clean Air	M	5th sternebra incomplete.
1213-107	1	Clean Air	F	5th sternebra incomplete.
	2	Clean Air	M	5th sternebra incomplete.
	10	Clean Air	M	5th sternebra incomplete.
1213-108	1	Clean Air	M	5th sternebra incomplete.
	2	Clean Air	M	5th sternebra incomplete.
	5	Clean Air	M	5th sternebra incomplete.
	7	Clean Air	M	5th sternebra incomplete.
1213-110	4	Clean Air	F	5th sternebra incomplete.
	6	Clean Air	F	5th sternebra incomplete.
	10	Clean Air	F	5th sternebra incomplete.
	11	Clean Air	M	1st sternebra incomplete. 2nd, 3rd and 4th sternebrae were malaligned. 6th sternebra appeared normal.
	13	Clean Air	M	5th sternebra incomplete. Left clav- icle was incomplete.

Continued...

TABLE 15 (Continued)
SKELETAL EXAMINATION OF FETUSES

<u>Dam Number</u>	<u>Fetus No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Abnormality</u>
1213-111	1	Clean Air	M	2nd and 5th sternebrae incomplete.
	4	Clean Air	F	5th and 6th sternebrae incomplete.
	8	Clean Air	F	5th sternebra incomplete.
	9	Clean Air	F	2nd and 5th sternebrae incomplete.
	10	Clean Air	M	5th sternebra incomplete.
	14	Clean Air	M	5th sternebra incomplete.
1213-124	3	Clean Air	F	5th sternebra incomplete.
1213-124	5	Clean Air	M	5th sternebra incomplete.
	8	Clean Air	F	5th sternebra incomplete.
1213-113	6	Diesel Exhaust	M	5th sternebra incomplete.
1213-114	6	Diesel Exhaust	M	Left clavicle only partially visible.
1213-116	8	Diesel Exhaust	M	5th sternebra incomplete.
	9	Diesel Exhaust	F	5th sternebra incomplete.
	12	Diesel Exhaust	M	5th and 6th sternebrae incomplete.
1213-117	2	Diesel Exhaust	M	5th sternebra incomplete.
	3	Diesel Exhaust	M	5th sternebra incomplete.
	5	Diesel Exhaust	F	5th sternebrae incomplete.
	9	Diesel Exhaust	F	5th and 6th sternebrae incomplete.

Continued...

TABLE 15 (Continued)

SKELETAL EXAMINATION OF FETUSES

<u>Dam Number</u>	<u>Fetus No.</u>	<u>Exposure Level</u>	<u>Sex</u>	<u>Abnormality</u>
1213-119	2	Diesel Exhaust	F	5th sternebra incomplete.
	7	Diesel Exhaust	M	5th sternebra incomplete.
	9	Diesel Exhaust	M	5th sternebra incomplete.
	10	Diesel Exhaust	M	5th sternebra incomplete.
1213-120	1	Diesel Exhaust	F	5th sternebra incomplete.
	11	Diesel Exhaust	F	5th sternebra incomplete.
1213-125	2	Diesel Exhaust	M	5th sternebra incomplete.
	3	Diesel Exhaust	F	5th sternebra incomplete.
	5	Diesel Exhaust	F	5th sternebra incomplete.
	6	Diesel Exhaust	F	5th sternebra incomplete.

TABLE 16
GROSS NECROPSY FINDINGS FOR 29-DAY LAPAROTOMY

<u>Dam Number</u>	<u>Group Number</u>	<u>Exposure Level</u>	<u>Gross Necropsy Findings</u>
1213-377	1	Clean Air	Right lung spotted diffusely and left lung abscessed severely. Liver showed small white abscesses. No other remarkable gross pathology was noted.
1213-380	1	Clean Air	All lobes of lung slightly reddened. No other remarkable gross pathology was noted.
1213-107	1	Clean Air	Lungs collapsed, pink with minute red foci (congestion). No other remarkable gross pathology noted.
1213-109	1	Clean Air	Lungs collapsed with focal terminal emphysematous areas pink and spongy. No other gross pathology was noted.
1213-124	1	Clean Air	Lungs collapsed, soft and dark red congestion. No other gross pathology was noted.
1213-383	2	Diesel Exhaust	Both lungs were diffused darkly. No other remarkable gross pathology noted.
1213-385	2	Diesel Exhaust	Lungs were pinpointed brown. No other remarkable gross pathology noted.
1213-386	2	Diesel Exhaust	Lungs appeared brown in color. No other remarkable gross pathology noted.
1213-387	2	Diesel Exhaust	Lungs were spotted diffusely. No other remarkable gross pathology was noted.
1213-388	2	Diesel Exhaust	Lungs were dark red in color. No other gross pathology was noted.
1213-389	2	Diesel Exhaust	Lungs were diffusely spotted with slight brown areas. No other gross pathology was noted.

Continued...

TABLE 16 (Continued)

GROSS NECROPSY FINDINGS FOR 29-DAY LAPAROTOMY

<u>Dam Number</u>	<u>Group Number</u>	<u>Exposure Level</u>	<u>Gross Necropsy Findings</u>
1213-390	2	Diesel Exhaust	Lungs were spotted with slight brown areas. No other remarkable gross pathology was noted.
1213-392	2	Diesel Exhaust	Lungs were diffused by brown areas. No other remarkable gross pathology was noted.
1213-393	2	Diesel Exhaust	All lobes of lungs were diffusely spotted brown. No other remarkable gross pathology was noted.
1213-394	2	Diesel Exhaust	Lungs were diffusely colored brown on all lobes. No other remarkable gross pathology was noted.
1213-112	2	Diesel Exhaust	Lungs collapsed, soft and tan with multiple black foci over the pleura. No other remarkable gross pathology was noted.
1213-115	2	Diesel Exhaust	Death due to toxemia resulting from intestinal strangulation through a traumatic vent in the left abdominal wall. No other remarkable gross pathology was noted.
1213-116	2	Diesel Exhaust	Lungs collapsed, pink with minute dark brown foci throughout. No other remarkable gross pathology was noted.
1213-117	2	Diesel Exhaust	Lungs collapsed, soft and diffusely tan. No other remarkable gross pathology was noted.
1213-118	2	Diesel Exhaust	Lungs collapsed, tan, spongy with multiple minute tan foci throughout. No other remarkable gross pathology was noted.
1213-125	2	Diesel Exhaust	Lungs collapsed and tan with multiple, irregular gray pleural foci. No other remarkable gross pathology was noted.

APPENDIX A

REQUEST FOR VETERINARY SERVICES

Date: January 24, 1979

Condition to be observed: Rabbit found dead at 8:00 A.M.

Animal Number: 1213-370

Study Number: WIL-1213-78

When observed: 8:00 A.M.

Room Number: 3

Observed by: Dr. Vincent Chaffee

Cage Position:

Professional Judgement: Animal necropsied and examined grossly. No tissues were saved for histopathological examination.

Action Required: This animal was necropsied 9:30 A.M. January 24, 1979 by Fred W. Sigler and grossly there was marked consolidation, "red hepatization" of the right diaphragmatic lobe of the lung with fibrinous adhesions to the thoracic wall. Also numerous petechiae over the pleura of the left diaphragmatic lobe of the lung.

Probable diagnosis: Pasteurellosis.

Signed: Vincent Chaffee, D.V.M.
Study Director

Signed: Fred W. Sigler, D.V.M., Pathologist
Date: January 24, 1979

APPENDIX B

REQUEST FOR VETERINARY SERVICES

Date: April 13, 1979

Condition to be observed: Dead rabbit

Animal Number: 1213-115

When observed: April 13, 1979

Observed by: Kathleen Werchowski

Study Number: WIL-1213-78

Room Number: Chamber 2 - EPA (Center Hill)

Cage Position: Top cage

Professional Judgement:

Action Required: Complete necropsy with handling of the fetuses in accordance with the protocol (WIL-1213-78).

This animal was necropsied at 4:00 P.M. April 13, 1979. The fur was oil soiled and beneath the skin on the left abdomen there was subcutaneous hemorrhage. When the abdomen was opened a 2 cm rent was found in the left abdominal wall, with a section of the jejunum herniated through this rent. This herniated loop of intestine was infarcted, soft and dark red. Also the mesenteric vessels were thrombosed.

The liver was friable with prominent lobular markings.

The lungs were inflated, dark red and firm. The trachial mucosa was congested.

Diagnosis: Death due to toxemia, resulting from intestinal infarction produced by traumatic herniation through the left abdominal wall.

Signed: Fred W. Sigler, D.V.M., Pathologist
Date: April 13, 1979

APPENDIX C

REQUEST FOR VETERINARY SERVICES

Date: February 20, 1979

Condition to be observed: Abscess on dewlap

Animal Number: 1213-395 (Male)

Study Number: WIL-1213-78

When observed: 2:00 P.M.

Room Number: 14

Observed by: Kathleen Werchowski

Cage Position: 3rd from right on bottom

Professional Judgement: Two large abscesses near mandibular lymph nodes.

Action Required: Surgical drainage and local application of organic iodine.

Vincent Chaffee
Study Director

Vincent Chaffee, D.V.M.
Date: February 20, 1979

APPENDIX D

REQUEST FOR VETERINARY SERVICES

Date: March 12, 1979

Condition to be observed: Abscesses in mandibular region

Animal Number: 1213-395

Study Number: WIL-1213-78

When observed: 9:00 A.M.

Room Number: 14

Observed by: Kathleen Werchowski

Cage Position: Bottom row

Professional Judgement: Animal does not have enough resistance to ward off continuous development of abscesses. Two new abscesses in submandibular area.

Action Required: Examine again on Wednesday.

Vincent Chaffee, D.V.M.

Date: March 12, 1979

APPENDIX E
REQUEST FOR VETERINARY SERVICES

Date: March 14, 1979

Condition to be observed:

Animal Number: 1213-395

When observed: 9:00 A.M.

Observed by: Kathleen Werchowski

Study Number: WIL-1213-78

Room Number: 14

Cage Position: Bottom row

Professional Judgement: The animal has a slight nasal discharge and is being watched for abscesses.